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Hyperinsulinism: molecular aetiology of focal disease

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Abstract
Persistent hypoglycaemia in infancy is most commonly caused by hyperinsulinism. A case is reported of the somatic loss of the maternal 11p in an insulin secreting focal adenoma in association with a germ-line SUR-1 mutation on the paternal allele in a baby boy with hyperinsulinism diagnosed at 49 days old. A reduction to homozygosiy of an SUR-1 mutation is proposed as a critical part of the cause of focal hyperinsulinism.

(Keywords: hyperinsulinism; sulphonylurea receptor; mutational analysis

Persistent hypoglycaemia in infancy is most commonly caused by hyperinsulinism. It is characterised by profound hypoglycaemia caused by inappropriate secretion of insulin. Hyperinsulinism may be inherited in both autosomal recessive and dominant forms. At the molecular level, mutations in the sulphonylurea receptor (SUR-1) and the potassium channel subunit (KIR6.2) have been identified in autosomal recessive forms of the disease. Somatic loss of maternal chromosome 11p has been demonstrated in non-familial cases with areas of focal adenomatosis and in adenomas of the pancreas. In these reports, the minimum region of chromosome 11p lost included the SUR-1 and KIR6.2 genes, as well as at least two maternally expressed imprinted genes (H19 and P57KIP2).

We report the somatic loss of the maternal 11p in an insulin secreting focal adenoma in association with a germline SUR-1 mutation on the paternal allele in a patient with hyperinsulinism. We propose a reduction to homozygosiy of an SUR-1 mutation as a critical part of the cause of focal hyperinsulinism.

Case report
A baby boy, with no family history of hypoglycaemia, presented with hypoglycaemia on day 42 of life. Hyperinsulinism was diagnosed at 49 days based on the finding of increased glucose requirement (15 mg/kg/min) to maintain euglycaemia. At a plasma glucose of 1.7 mmol/l the patient’s insulin concentration was 297 pmol/l (normal < 14 pmol/l); at a plasma glucose of 0.4 mmol/l, insulin was 75 pmol/l, 3-hydroxybutyrate was suppressed at 0.02 mmol/l (normal > 1.5 mmol/l), and the patient had a glycaemic response to glucagon of > 1.7 mmol/l. Diazoxide and octreotide treatment was unsuccessful and surgery was performed on day 84.

At surgery an adenoma was palpated and partial pancreatectomy (approximately 40%) performed. Histology confirmed an insulin staining adenoma with normal pancreatic tissue in the surrounding area. The patient recovered well, tolerated a 13 hour fasting interval at age 4 months, and has had no subsequent hypoglycaemia.

Genomic DNA was extracted from patient and parental peripheral blood leucocytes, and from patient paraffin embedded tissue using standard techniques. SUR-1 exon 10 sequences were amplified using standard polymerase chain reaction (PCR) conditions (GibcoBRL; Life Technologies, Paisley, UK) using exon 10 primers SUR-10F (5’-GTGGAGACGCCGCA GGAAGGAG–3’) and SUR-10R (5’–CCCT GCATGTACGCAGCAGCACCC–3’). Equal quantities of PCR product was digested for at least two hours with BsrI using conditions as described by the manufacturer. BsrI digestion of wild-type product yields two fragments of 66 and 50 base pairs. The mutated allele remains uncut. For GATA23FO6 and TH’ microsatellite analysis, PCR was performed as above and analysed on a 7% Long Ranger gel run on a Pharmacia ALF automatic sequencing system (Pharmacia BioFech, St Albans, UK).

Results
Patient leucocyte DNA was screened for 24 previously identified SUR-1 mutations and 2 KIR6.2 mutations; heterozygosity for an SUR 1 intron 10 splice site mutation (1630+1 G→T) was discovered. This mutation had previously been identified in three other patients with hyperinsulinism. One was a compound heterozygote for this and another SUR-1 mutation, whereas in the other two the mutation was identified on the paternal allele with no mutation identified on the maternal allele. As this mutation destroys a BsrI restriction site, enzyme digestion of exon 10 PCR product was used to identify the mutation in patient and parental samples (fig 1), demonstrating that the mutation was paternally derived. Analysis of DNA from the patient’s normal pancreatic tissue demonstrated a heterozygous pattern of normal and mutant allele. In contrast, DNA extracted from adenoma tissue contained mostly mutant allele.

To investigate if this was the result of loss of the maternal allele we performed microsatellite analysis using 11p specific markers GATA23FO6 and TH. TH lies approximately
30 centimorgans telomeric to the SUR-1 gene with the GATA23FO6 between the TH and SUR-1. The results demonstrated a Mendelian inheritance pattern in the patient’s leucocyte and normal pancreatic tissue, and a loss of the maternal 11p in the adenoma (fig 2). Our results indicate that the hyperinsulinism in this case is as a result of a reduction to homozygosity of the SUR-1 mutation in the adenoma tissue.

Discussion

de Lonlay and colleagues demonstrated loss of heterozygosity (LOH) in focal hyperplasia of the pancreas. They proposed that the hyperplasia was either caused by a loss of the imprinted tumour suppresser genes H19 or P57KIP2, or an expression imbalance between H19 and IGF II. Their studies explain the hyperplasia in focal disease but do not adequately address the hypersecretion of insulin in the focal tissue. Beckwith-Wiedemann syndrome, a condition in which 20% have LOH of chromosome 11p (the region containing the SUR-1 locus) is associated with hyperinsulinism in 30% of cases, but the occurrence of hyperinsulinism in these patients does not correlate with the occurrence of the LOH. We propose a model where the somatic loss of the maternal 11p is the causative factor for focal hyperplasia but that the hyperplasia itself is not sufficient to cause insulin hypersecretion. With the additional presence of a paternally derived SUR-1 mutation, there is a reduction to homozygosity for SUR-1 in focal cells resulting in dysregulation of insulin secretion and ultimately to the hyperinsulinism phenotype. Resection of the affected tissue resulted in a cure in a similar fashion to the partial pancreatectomy performed by de Lonlay et al in focal hyperplasia, unlike autosomal recessive hyperinsulinism in which a 95% pancreatectomy is often insufficient. In conclusion, this case demonstrates a novel mechanism for the apparent sporadic inheritance of disease considered an autosomal recessive disorder.
Acute scrotal swelling

Beware the facts that everybody knows. Everybody knows, for instance, and paediatric textbooks confirm, that epididymitis is rare in children. Several case series, however, have suggested that this may not be true. In Salt Lake City, USA 64 of 90 boys with acute scrotal pain or swelling had epididymitis and 30 of the 64 were under 12 years old (Howard A Kadish and Robert G Bolte. Pediatrics 1998;102:73–6). Of the remaining 26, 13 had testicular torsion (TT) and 13 torsion of the appendix testis (TAT). Fewer of those with epididymitis presented within 12 hours of symptom onset and they were more likely than those with torsion to have fever, a history of previous similar pain, or dysuria, although each of these features was present in fewer than 20% of boys with epididymitis. The testicular lie was normal in all boys with epididymitis or TAT but abnormal in almost half of those with TT. The testis as a whole was tender in all those with TT, 69% of those with epididymitis, and 31% of those with TAT, but all boys with TAT had localised tenderness at the superior pole of the testis. The cremasteric reflex was absent in all patients with TT, 14% with epididymitis, and none with TAT. Scrotal erythema or oedema was more common in epididymitis (67%) than in TT (38%) or TAT (8%, one patient). Coloured Doppler ultrasound showed decreased blood flow in all seven patients with TT in whom it was performed, one of 29 with epididymitis, and neither of two with TAT. Ten of the 13 boys with TT had salvageable testes.

None of this should lead clinicians into delaying surgery for boys who might have testicular torsion. In Salt Lake City they operate right away if the diagnosis is at all likely and do Doppler studies only when there is serious doubt.